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Byung-Kuk Yoo, Isabelle Lamarre, Jean-Louis Martin, Fabrice Rappaport, Michel Negrierie. Dynamics of NO interacting with soluble guanylate cyclase from 1 ps to 0.1 s and induced structural transitions. BMC Pharmacology, 2011, 11 (Suppl 1), pp.P77. inserm-00612922

HAL Id: inserm-00612922

<https://www.hal.inserm.fr/inserm-00612922>

Submitted on 1 Aug 2011

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POSTER PRESENTATION

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Dynamics of NO interacting with soluble guanylate cyclase from 1 ps to 0.1 s and induced structural transitions

Byung-Kuk Yoo¹, Isabelle Lamarre¹, Jean-Louis Martin¹, Fabrice Rappaport², Michel Negrier^{1*}

From 5th International Conference on cGMP: Generators, Effectors and Therapeutic Implications
Halle, Germany. 24-26 June 2011

Results

We investigated the interaction between purified soluble guanylate cyclase (sGC) from beef lung and NO by time-resolved spectroscopy in a time-range which encompasses eleven orders of magnitude, from 1 ps [1] to 0.1 s [2]. After its dissociation from the heme, NO either recombines geminately to the 4-coordinate heme within $\tau_{G1} = 7$ ps [1] (96 ± 1 % of the population) or exits the heme pocket (4 ± 1 %), allowing the proximal histidine to rebind within 62 ± 10 ps. Then, NO is distributed in two approximately equal populations (~ 2 %). One geminately rebinds to the 5-coordinate heme ($\tau_{G2} = 6.5$ ns) while the other migrates into the solution. NO can rebind from the solution (bimolecular rebinding, $\tau_B = 0.25$ ms with $[NO] = 20$ μM), forming a 6-coordinate heme with a rate constant of 2×10^8 $M^{-1} s^{-1}$ (*in vitro* purified protein) very close to that measured in platelets (3×10^8 $M^{-1} s^{-1}$) [3].

The cleavage of Fe-His bond and subsequent formation of 5-coordinate NO-heme occurs with different

time constants for NO which geminately rebinds ($\tau_{5C1} = 0.66$ μs) and for NO which binds from the solution ($\tau_{5C2} = 43$ ms). Thus, because the same structural event occurs with rates separated by more than 4 orders of magnitude, we must infer that sGC is not in the same structural state in both cases, with a different strain exerted on the Fe-His bond. This allosteric transition between both states occurs in the time range 0.66 $\mu s < \tau_R < 250$ μs in sGC after His rebinding and NO release.

Conclusion

Since the discovery that NO binds to the proximal heme side in cytochrome c' [4] (AXCP), several models of sGC activation were proposed which include the binding of NO to the proximal heme side despite the lack of observation for such an activation step in sGC. After the fast histidine rebinding in the picosecond range, we have observed only four phases in the nano to millisecond time range (assigned as indicated in Table 1). Thus,

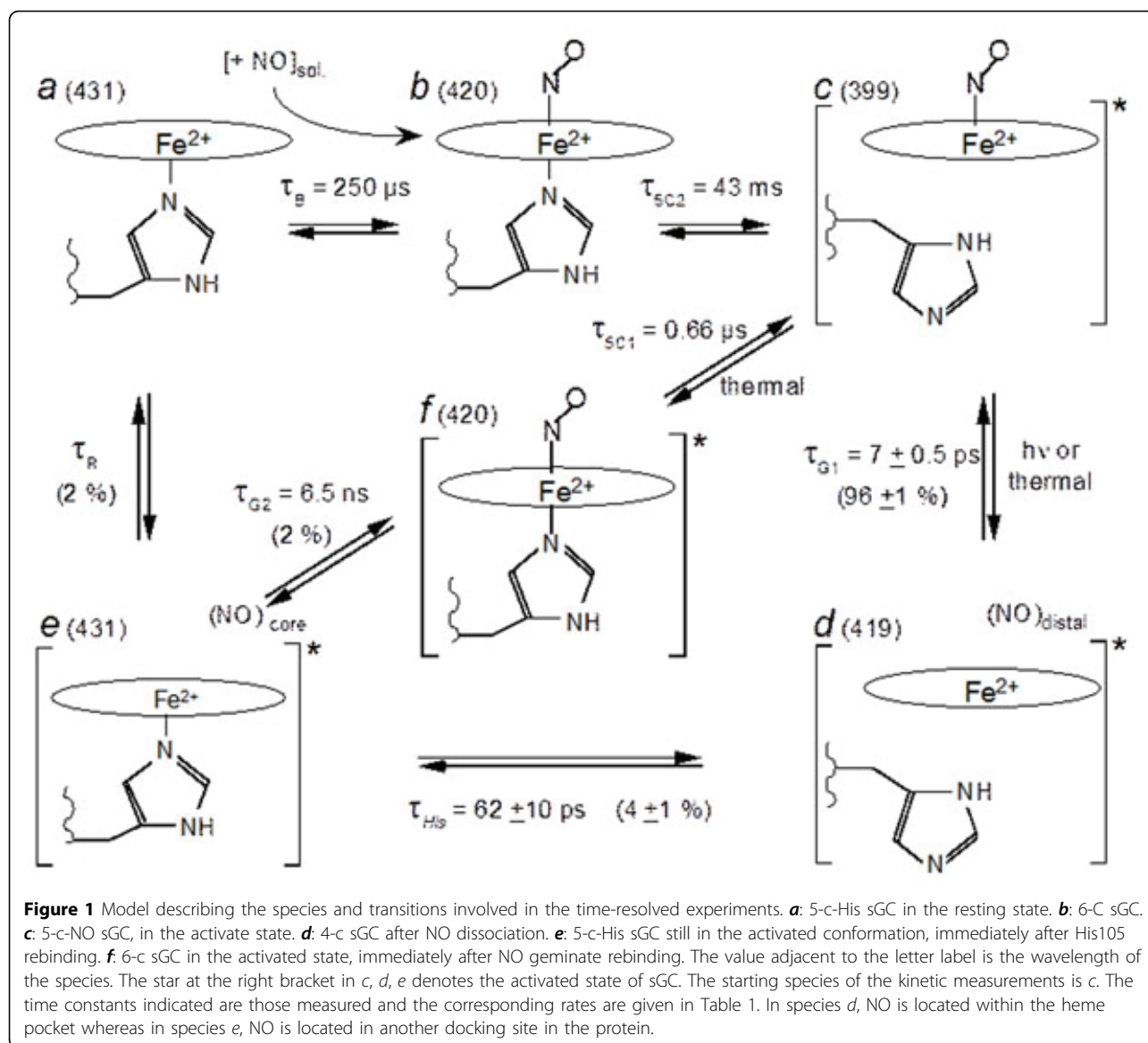
Table 1 Rates of the transitions observed in kinetics.

Transition	Time constants	Transition rates
Bimolecular NO binding to 5c-His	$\tau_B = 0.25$ ms; $[NO] = 20$ μM	$k_B = 2 \times 10^8$ $M^{-1} s^{-1}$
Conversion 6c-NO \rightarrow 5c-NO	$\tau_{5C2} = 43$ ms	$k_{5C2} = 23$ s^{-1}
Geminate NO rebinding to 5c-His	$\tau_{G2} = 6.5$ ns	$k_{G2} = 0.15 \times 10^9$ s^{-1}
Conversion 6c*-NO \rightarrow 5c*-NO	$\tau_{5C1} = 0.66$ μs	$k_{5C1} = 1.5 \times 10^6$ s^{-1}
His rebinding to 4c-heme	$\tau_{His} = 62$ ps	$k_{His} = 1.4 \times 10^{10}$ s^{-1}
Geminate NO rebinding to 4c-heme	$\tau_{G1} = 7$ ps	$k_{G1} = 0.13 \times 10^{12}$ s^{-1}
Structural relaxation sGC* \rightarrow sGC	0.66 $\mu s < \tau_R < 250$ μs	4×10^3 $s^{-1} < k_R < 1.5 \times 10^6$ s^{-1}

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analysis of the entire NO dynamics from 1 ps to 0.1 s did not detect NO binding to the proximal side of sGC heme despite the fact that NO shows the same geminate rebinding to the 4-coordinate heme in sGC and AXCP [5]. Our data can be described with a one-site model, without phases assigned to dinitrosyl formation or to NO proximal binding.

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Published: 1 August 2011

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doi:10.1186/1471-2210-11-S1-P77

Cite this article as: Yoo et al.: Dynamics of NO interacting with soluble guanylate cyclase from 1 ps to 0.1 s and induced structural transitions. *BMC Pharmacology* 2011 11(Suppl 1):P77.